

THE AMENDMENTS

In The Specification:

Please amend the paragraph starting at page 23, line 25:

The hybridization mixture is heated to 95°C and afterwards, equilibrated to 37°C. After the boiling procedure, the smears are incubated with each 50 µl of the hybridization mixture for 4 hours at 42°C. The samples are washed in excess volumes of the wash buffers two times in 2 x SSC at 37°C for 15 min, and once in 1 x SSC at 37 °C for 15 min. Then, the smears are rinsed two times at room temperature in 2 x SSC. Following this washing procedure, the dissections are incubated for 30 min with blocking buffer (NEN, blocking buffer) at room temperature, followed by a 1 hour incubation with a 1:100 diluted (in blocking buffer, see above) Streptavidin-alkaline phosphatase and monoclonal mouse HRP-labeled anti-Digoxigenine anti-digoxigenine antibodies (Molecular Probes). The smears are then washed 2 times in 1 x PBS/0.1% Triton TRITON® X-100 (polyethylene glycol tert-octyl-phenyl ether) for 10 min at room temperature, followed by one wash step with 1 x PBS, 50 mM MgCl₂ (pH 9.2) for 10 min at room temperature. Then the staining reaction is performed with ELF 97 phosphate (Molecular Probes) for 10 sec to 7 min at room temperature. Excess substrate is washed 3 times with 1x PBS/0.1% Triton TRITON® X-100 for 10 min at room temperature. In a second staining step, the section is incubated with Tyramides-Alexa-Fluor 594 for 10 sec to 7 min. Excess substrate is washed 3 times with 1x PBS/0.1% Triton TRITON® X-100 for 10 min at room temperature. Finally the smears are dipped in H₂O_{dest.} and embedded with Fluorescence mounting medium (DakoCytomation). Then the stained dissections can be analysed by fluorescence microscopy.

Please amend the paragraph starting at page 24, line 27:

~~Merkofix~~® MERCKOFIX® (spray-fixative) fixed cytological samples (conventional smears and liquid-based cytology (ThinPreps THINPREP®)) of the cervix uteri are immunofluorescent stained using antibodies specific for p16^{INK4a} and Ki-S2.

Please amend the paragraph starting at page 25, line 1:

Conventional smears and liquid based cytological samples (ThinPreps THINPREP®) are rehydrated in ethanol (50%) for 10min and transferred in Aqua bidest. Antigen retrieval is

carried out with 10mM citrate buffer (~~pH 6.0~~) (pH 6.0) for p16^{INK4a} and Ki67. Therefore, the slides are heated in a waterbath for 40 min at 95 ° – 98°C. The slides are cooled down to RT for 20 minutes, transferred to washing buffer (50mM Tris-HCl, 150mM NaCl, 0.05% ~~Tween~~ TWEEN® 20 (polyethylene glycol sorbitan monolaurate)/ DakoCytomation: code no.: S3006), and finally, the samples are surrounded with a lipid-pencil.

Please amend the paragraph starting at page 25, line 9:

The slides are then incubated with the primary antibodies, mouse anti-human p16^{INK4a} antibody (clone E6H4) (3.48 µg/ml) and rabbit anti-Ki-S2 (1:25) for 30 min at RT, and then the slides are rinsed with washing buffer and placed in a fresh buffer bath for 5 min. Excess buffer is tapped off, and the specimen is covered with 200µl of the secondary reagent containing goat anti-mouse antibody, ~~AlexaFluor~~® ALEXA FLUOR® (fluorescent chemical label) 488 conjugated and goat anti rabbit antibody, and ~~AlexaFluor~~® ALEXA FLUOR® 546 conjugated and then incubated for 30min at RT. Then, slides are washed two times as before and directly mounted with a special mounting medium for fluorescence.

Please amend the paragraph starting at page 26, line 4:

Cells contained in bronchioalveolar lavage specimens of patients are prepared according to ~~ThinPreps~~ THINPREP® technology. ~~Merckofix~~® MERCKOFIX®-fixed cytological samples of the lavages of patients diagnosed with small cell lung cancer are immunofluorescent stained using antibodies specific for p16^{INK4a}, Ki67, and PCNA.

Please amend the paragraph starting at page 26, line 20:

The tissue sections are rehydrated through incubation in xylene and graded ethanol, and then transferred to Aqua bidest. Conventional smears and liquid-based cytological samples (~~ThinPreps~~ THINPREP®) are rehydrated in ethanol (50%) for 10min, and transferred in Aqua bidest. Antigen retrieval is carried out with 10mM citrate buffer (pH 6.0) for p16^{INK4a}, Ki67, and PCNA. Therefore, the slides are heated in a waterbath for 40 min at 95 - 98°C. The slides are cooled down to RT for 20 minutes, transferred to washing buffer (50mM Tris-HCl, 150mM

NaCl, 0.05% Tween 20 / DakoCytomation: code no.: S3006), and finally, the samples are surrounded with a lipid-pencil.

Please amend the paragraph starting at page 27, line 25:

Cells were centrifuged, the supernatant decanted and fixed and permeabilized with 100 ml ~~Permeafix~~ PERMEAFIX[®] (permeabilization and fixation agent, Ortho Diagnostic, Raitan, NJ, USA) for 1 hour at room temperature. Cells were washed in sterile PBS, pH 7.4, pelleted, and re-suspended in 100 ml ~~Permeafix~~ PERMEAFIX[®] for 1 hour at room temperature. Cells were washed in sterile PBS, pH 7.4, pelleted, and re-suspended in sterile PBS. They were incubated with PE-conjugated anti-p14ARF antibody and PE-Cy5-conjugated anti-Ki67 antibody for 1 h at +4°C. Cells were washed in sterile PBS, pH 7.4, pelleted, and re-suspended in 100 ml Permeafix for 30 min at room temperature. Cells were washed in sterile PBS, pelleted by centrifugation, and then washed again in 2x standard saline citrate (SSC). After centrifugation, the cell pellet was resuspended in hybridization solution (2x SSC, 30% formamide, sonicated salmon sperm, and yeast transfer DNA) containing 500 ng of 5-carboxy-fluorescein double end-labelled, mcm5-specific oligonucleotides probes. The intercellular hybridization was performed at 42°C for 1 hour, followed by successive washes in 2x SSC, 0.5 % ~~Triton~~ TRITON[®] X-100, and 1x SSC, 0.5 % ~~Triton~~ TRITON[®] X-100 at 42°C. The cells were re-suspended for analysis in PBS, pH 8.3, and analyzed on a flow cytometer (FACScan, Becton Dickinson, IS). For each analysis, ~~30,000-100,000~~ 30,000-100,000 gated events were collected. Data analysis was performed using CellQuest software (Becton Dickinson, IS).